DATA EVALUATION RECORD

1. **CHEMICAL:** Bromoxynil octanoate
Shaughnessey Number 035301.

2. **TEST MATERIAL:** Bromoxynil Octanoate Technical; 2,6-dibromo-4-cyanophenyl octanoate; M & B Lot No. CN-51033 (20-DLM-152-1); Analytical Log No. 14542; 97.2% active ingredient; a brown solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants - Tier II. Species Tested: Selenastrum capricornutum.

4. **CITATION:** Giddings, J.M. 1990. Bromoxynil Octanoate - Toxicity to the Freshwater Green Alga Selenastrum capricornutum. Prepared by Springborn Laboratories, Inc., Wareham, Massachusetts. SLI Report #90-8-3436. SLI Study #10566-1089-6142-430. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, North Carolina. MRID Number 416060-04.

5. **REVIEWED BY:**

Kimberly Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Kimberly Rhodes
Date: February 11, 1991
Chuck Jass 2/15/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: P. Kosalwat
Date: 2/12/91

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 2/19/91

7. **CONCLUSIONS:** This study is NOT scientifically sound ~~but~~ AND does not fulfill the guideline requirements for a Tier II growth and reproduction test using a non-target aquatic plant. Due to the inconsistency of the measured concentrations, the actual exposure concentrations of this test are not known. With a 5-day EC50 value of 0.21 mg/L mean measured concentration, Bromoxynil is expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) when applied at application rates up to 0.375 lbs a.i./A.

Upgraded to core

7 hrs

8. **RECOMMENDATIONS:** Tier III testing should be conducted since Bromoxynil is expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) at the maximum application rate.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Organism:** The freshwater green alga (Selenastrum capricornutum) used in this toxicity test was originally obtained from Carolina Biological Supply Company located in Burlington, North Carolina. The stock culture was maintained under test conditions at the testing facility.

Stock cultures were transferred to fresh medium approximately once or twice a week. The inoculum used to initiate the toxicity test with Bromoxynil was taken from a stock culture that had been transferred to fresh medium four days before testing.

The culture medium used was Marine Biological Laboratory (MBL) medium prepared with deionized water and adjusted to pH 7.5 ± 0.1 with 0.1N hydrochloric acid after autoclaving. Stock cultures were grown in 125-mL glass flasks containing 50 mL of medium. The flasks were covered with stainless steel caps which permitted gas exchange.

B. **Test System:** The phytotoxicity test was conducted in an environmental chamber where the temperature was maintained at 22-26°C. The test vessels were sterile 125-mL flasks fitted with stainless steel caps which permitted gas exchange. The flasks were impartially placed on an orbital shaker set at 100 rpm. Lighting was provided continuously at an intensity of 4,000-5,000 lux at the solution surface.

The test medium used to prepare the exposure solutions was formulated in the same manner as the culture medium (excluding Na_4EDTA).

C. **Dosage:** Five-day growth and reproduction test. The nominal test concentrations of Bromoxynil based on active ingredient were 0.016, 0.031, 0.063, 0.13, 0.25, 0.50, and 1.0 mg/L.

D. **Design:** Based on the results of preliminary testing, a control, solvent control, and seven nominal Bromoxynil concentrations (see Section 11.C) were selected for testing. The solvent control contained 0.1 mL/L of acetone which was equivalent to the concentration of solvent present in all test solutions. Each concentration and control were replicated three times.

After the test solutions were added to the test flasks, an inoculum of Selenastrum capricornutum cells calculated to provide 0.3 x 10^4 cells/mL was aseptically introduced into each flask. The inoculum volume was 780 µL per flask. At each 24-hour interval, cell counts were conducted on each replicate vessel using a hemacytometer and a compound microscope. One sample was taken from each flask for counting. One or more hemacytometer fields, each 0.1 x 0.1 cm in surface area and 0.01 cm deep and containing 0.0001 mL of culture, were examined for each sample until at least 400 cells or four fields were counted; when cell densities were less than 100 x 10^4 cells/mL, fewer than 400 cells were present in four fields but no more than four fields were counted.

Water quality parameters (pH and conductivity) were measured at test initiation and termination. Measurements at test initiation were conducted on the test solutions remaining in the 500-mL volumetric flasks after the test flasks had been filled. At test termination, the remaining test solution in each replicate of each test concentration were composited and a portion of the composite solution was transferred to a 100-mL beaker for pH and conductivity measurement. Temperature was measured continuously with a minimum/maximum thermometer in a flask of water placed next to the test vessels. The shaking rate of the orbit shakers was recorded daily. The light intensity of the test area was measured with a light meter at test initiation and each 24-hour interval of the exposure period.

At test initiation, samples for analysis were removed from the 500-mL volumetric flasks of the test solutions and the controls and frozen for analysis. In addition, six quality control (QC) samples were prepared. The results of the analysis of these QC samples were used to judge the precision and quality control maintained during the analytical process. All solutions were

analyzed for Bromoxynil by high pressure liquid chromatography.

E. **Statistics:** EC10, EC50, and EC90 values and their 95% confidence limits were determined after 96 and 120 hours of exposure by linear regression of response (percent reduction of cell density as compared with controls) vs. mean measured exposure concentration. Four linear regressions were estimated based on (a) untransformed data, (b) untransformed response vs. logarithm-transformed concentration, (c) probit-transformed response vs. untransformed concentration, and (d) probit-transformed response vs. logarithm-transformed concentration. The regression that best fitted the data was selected based on the highest coefficient of determination (r^2). This regression equation was then applied to estimate the EC values and their 95% confidence limits, using the method of inverse prediction.

A t-test was used to compare the controls with solvent controls. Comparison of controls with solvent controls indicated no significant difference (P = 0.01) in cell density. The data from the two sets of controls were therefore pooled for analysis. Before conducting the analysis, the data were checked for normality using the Chi-Square test and for homogeneity of variance using Hartley's Test. The no-observed-effect concentration (NOEC) was determined using one-way analysis of variance and Bonferroni's Test since treatment groups had unequal numbers of replicates (i.e., control data were pooled).

12. **REPORTED RESULTS:** The mean measured concentrations of Bromoxynil were 0.016, 0.018, 0.041, 0.088, 0.18, 0.26, and 0.53 mg/L (Table 2, attached). The mean measured concentrations ranged from 51 to 71 percent of the nominal concentrations. At test termination, measured concentrations averaged 50% of nominal concentrations. Possible causes of the decline in Bromoxynil concentration include uptake or sorption by algae, removal by physical or chemical processes (e.g., photolysis or hydrolysis) and/or biological degradation.

The measured concentration in the lowest nominal treatment level tested (0.016 mg a.i./L) was below detection limits at 120 hours. The detection limit was 83% of the nominal concentration for this test solution. For this treatment level, the test concentration was defined based on initial

measured concentration rather than on mean measured concentration. Recovery of Bromoxynil from QC samples averaged 100% at 0 hours and 93% at 120 hours.

Cell densities determined at each observation time are presented in Table 3 (attached). Cell densities increased over time in all replicates and generally followed the concentration gradient established. Cells in cultures exposed to 0.088-0.53 mg/L mean measured concentrations were bloated and fragmented, and at test termination were less curled than normal. Cells in cultures exposed to 0.041 mg/L mean measured concentration were somewhat clumped on some observation days. Other cultures appeared normal. Control densities averaged 119 x 10^4 cells/mL at 120 hours. Solvent control densities averaged 121 x 10^4 cells/mL at 120 hours.

Cell densities at the three highest mean measured concentrations (0.18, 0.26, and 0.53 mg/L) averaged 50%, 44% and 46% of the pooled controls, respectively, at test termination. The 120-hour EC10 value, based on mean measured concentrations, was determined to be 0.0087 mg/L with a 95% confidence interval of 0.00056-0.071 mg/L. The 120-hour EC50 value, based on mean measured concentrations, was determined to be 0.22 mg/L with a 95% confidence interval of 0.026-2.5 mg/L. The 120-hour EC90 value, based on mean measured concentrations, was determined to be 5.5 mg/L with a 95% confidence interval of 0.60-172 mg/L. The 120-hour NOEC was determined by Bonferroni's test to be 0.016 mg/L initial measured concentration.

At test initiation, conductivity was 220 µmhos/cm and pH ranged from 7.4 to 7.5. At test termination, conductivity ranged from 230 to 310 µmhos/cm and the pH had increased to 10.0 to 10.1. Temperatures ranged from 22 to 26°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the author.

The study was conducted following the intent of the Good Laboratory Practice Regulations and the study conduct, raw data and final report were reviewed by Springborn Laboratories, Inc. Quality Assurance Unit. A Quality Assurance Statement was included and signed by the Quality Assurance Manager.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviation:

o The SEP states that a light intensity of 4,000 lux should be provided continuously. During this study, the light intensity ranged from 4,000 to 5,000 lux.

B. Statistical Analysis: The reviewer used the EPA's Toxanal computer program to calculate the 5-day EC50 value using cell density percent inhibition as the growth endpoint. These calculations are attached. The EC50 value was calculated using only the six highest mean measured concentrations since the lowest concentration (0.016 mg/L) was slightly stimulatory (2%) relative to the solvent control. Percent inhibition (I) of growth compared to the solvent control was calculated for cell density according to the following formula:

$$\% I = \frac{C - T}{C} \times 100$$

where: C = mean growth in the control,
T = mean growth in test concentration.

The 5-day EC50 value, based on mean measured concentrations, was determined to be 0.21 mg/L with a 95 percent confidence interval of 0.12-0.50 mg/L. The slope of the concentration-response curve was determined by probit analysis to be 0.44.

Analysis of variance with multiple comparison tests was performed to compare cell density at each treatment level to those of the control and solvent control for day 5 (attached). The 5-day NOEC value was determined to be 0.016 mg/L initial measured concentration.

C. Discussion/Results: This study is scientifically sound but does not fulfill the guideline requirements for a Tier II growth and reproduction test using a non-target aquatic plant. Measured concentrations of the test solutions at 0 hour and 120 hours are inconsistent (Table 2, attached). Concentrations in one test level (0.13 mg a.i./L nominal concentration) increased from 0 hour to 120 hours, while the other test levels significantly decreased. There also appears to be a problem with the solubility of the test material since

the initial measured concentrations ranged from 66 to 98% of the nominal values and the 120-hour measured concentrations ranged from 30 to 70% of the nominal concentrations. Since the water samples were not filtered before chemical analysis, some samples might have contained precipitates or particles which contributed to the inconsistency of measured concentrations observed. Therefore, the actual exposure concentrations in this test are not known. The 5-day EC50 value of Bromoxynil for Selenastrum capricornutum was determined to be 0.21 mg/L mean measured concentration.

Direct application of 0.375 lb a.i./acre (A) to a one acre, 0.5 feet deep pond would result in an estimated environmental concentration (EEC) of 0.275 mg a.i./L, which is slightly higher than the estimated 5-day EC50 value of 0.21 mg/L mean measured concentration. Therefore, Bromoxynil is expected to exert a detrimental effect on the green alga (Selenastrum capricornutum) following normal application methods at rates up to 0.375 lbs a.i./A. Based on these results a Tier III toxicity test is required.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Invalid~~. Upgraded to core (See D170117)

(2) **Rationale:** Actual exposure concentrations are not known.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 01-18-91.

Page_____ is not included in this copy.

Pages 8 through 10 are not included.

The material not included contains the following type of information:

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5-day EC50

KIMBERLY RHODES BROMOXYNIL SSELENASTRUM CAPRICORNUTUM 01-18-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.53	100	55	55	0
.26	100	57	57	0
.18	100	50	50	0
.088	100	37	37	0
.041	100	39	39	0
.018	100	33	33	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .18

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.4409999	.177701	.1124964	.5105699

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2	.2206035	1	.5311586

SLOPE = .4415663

95 PERCENT CONFIDENCE LIMITS = .2341695 AND .6489631

LC50 = .207386

95 PERCENT CONFIDENCE LIMITS = (.1219839 AND .5018352)

LC10 = 2.758381E-04

95 PERCENT CONFIDENCE LIMITS = 1.29359E-06 AND 1.970956E-03

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5-day NOEC

Analysis of Variance File: BROMNOEC Date: 01-18-1989

FILTER: None

N's, means and standard deviations based on dependent variable: CELLDEN

* Indicates statistics are collapsed over this factor

	Factors: C	Mean Measured Concentrations (mg/L)	N	Mean	S.D.
	*		27	84.5185	28.5703
	1	Solvent Control	3	120.6667	10.2632
	2	Control	3	118.6667	13.5031
	3	0.016	3	123.0000	12.7671
*	4	0.018	3	80.6667	8.5049
*	5	0.041	3	73.6667	3.2146
*	6	0.088	3	76.3333	4.9329
*	7	0.18	3	60.0000	2.6458
*	8	0.26	3	52.6667	7.6376
*	9	0.53	3	55.0000	5.2915

Fmax for testing homogeneity of between subjects variances: 26.05
Number of variances= 9 df per variance= 2.

Analysis of Variance Dependent variable: CELLDEN

Source	df	SS (H)	MSS	F	P
Between Subjects	26	21222.7402			
C (CONC)	8	19920.7422	2490.0928	34.425	0.0000
Subj w Groups	18	1301.9981	72.3332		

* - indicates significant effect (p=0.01)

[redacted]

Shaughnessy No. 035301 Chemical Name Bromoxynil Octanoate Chemical Class _____ Page ____ of ____

Study/Species/Lab/ Accession / Chemical I.a.i. _____ Results _____ Reviewer/ Date _____ Validati Status

14-Day Single Dose Oral LD_{50}

Species _____ LD50 = _____ mg/kg (95% C.L.) Contr. Mort.(%)= _____

Lab _____ Slope= _____ * Animals/Level= _____ Age(Days)= _____

Acc. _____ 14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ) Sex = _____

Comments:

14-Day Single Dose Oral LD_{50}

Species _____ LD50 = _____ mg/kg. (95% C.L.) Contr. Mort.(%)= _____

Lab _____ Slope= _____ * Animals/Level= _____ Age(Days)= _____

Acc. _____ 14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ) Sex = _____

Comments:

8-Day Dietary LC_{50}

Species _____ LC50 = _____ ppm (95% C.L.) Contr. Mort.(%)= _____

Lab _____ Slope= _____ * Animals/Level= _____ Age(Days)= _____

Acc. _____ 8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ) Sex = _____

Comments:

8-Day Dietary LC_{50}

Species _____ LC50 = _____ ppm (95% C.L.) Contr. Mort

Lab _____ Slope= _____ * Animals/Level= _____ A

Acc. _____ 8-Day Dose Level ppm/(%Mortality) ( ),

Comments:

48-Hour LC_{50}

Species _____ LC50 = _____ PP_ (95% C.L.)

Lab _____ Slope= _____ * Animals/Level=

Acc. _____ 48-Hour Dose Level pp ( ),

Comments:

Species _____ LC50 = _____ PP_ (95

Lab _____ Slope= _____ * Ani

96-Hour Dose

( ),

Comments:

5-day NOEC

Analysis of Variance File: BROMNOEC Date: 01-18-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	120.667	6	76.333
2	118.667	7	60.000
3	123.000	8	52.667
4	80.667	9	55.000
5	73.667		

	Comparison	Tukey-A*	Newman -Keuls*	Bon-ferroni	Dunnett
	1 > 2				
	1 < 3				
**	1 > 4	0.0100	0.0100	0.0008	0.0100
**	1 > 5	0.0100	0.0100	0.0000	0.0100
**	1 > 6	0.0100	0.0100	0.0000	0.0100
**	1 > 7	0.0100	0.0100	0.0000	0.0100
**	1 > 8	0.0100	0.0100	0.0000	0.0100
**	1 > 9	0.0100	0.0100	0.0000	0.0100
	2 < 3				N.A.
	2 > 4	0.0100	0.0100	0.0015	N.A.
	2 > 5	0.0100	0.0100	0.0000	N.A.
	2 > 6	0.0100	0.0100	0.0000	N.A.
	2 > 7	0.0100	0.0100	0.0000	N.A.
	2 > 8	0.0100	0.0100	0.0000	N.A.
	2 > 9	0.0100	0.0100	0.0000	N.A.
	3 > 4	0.0100	0.0100	0.0000	N.A.
	3 > 5	0.0100	0.0100	0.0000	N.A.
	3 > 6	0.0100	0.0100	0.0000	N.A.
	3 > 7	0.0100	0.0100	0.0000	N.A.
	3 > 8	0.0100	0.0100	0.0000	N.A.
	3 > 9	0.0100	0.0100	0.0000	N.A.
	4 > 5				N.A.
	4 > 6				N.A.
	4 > 7		0.0500		N.A.
	4 > 8	0.0500	0.0100	0.0289	N.A.
	4 > 9	0.0500	0.0500	0.0604	N.A.
	5 < 6				N.A.
	5 > 7		0.1000		N.A.
	5 > 8		0.0500		N.A.
	5 > 9		0.0500		N.A.
	6 > 7		0.1000		N.A.
	6 > 8	0.1000	0.0500		N.A.
	6 > 9		0.0500		N.A.
	7 > 8				N.A.
	7 > 9				N.A.
	8 < 9				N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000).
A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible
and only for comparisons with the control mean (level 1).

** indicates a significant effect.